channel current (I_{Ca}) recovery from inactivation was measured (τ ~ 53ms, V_{hold} = −80mV) and found to be much faster than CaTR; suggesting a minimal role. Interestingly, decreasing RyR cytosolic Ca^{2+} sensitivity with tetracaine strongly shifted CaTR curve to the right. While increasing RyR Ca^{2+} sensitivity with caffeine only moderately shifted the CaTR curve to the left. These results were then compared to $[Ca^{2+}]_{SR}$ measurements under similar conditions and times. The roles of RyR state and SR Ca^{2+} load in determining CaTR will be discussed. These findings are relevant to an investigation of the mechanisms of Ca^{2+} signaling instability and SR Ca^{2+} leak in arrhythmic conditions including those attributable to myocardial infarction, heart failure and inheritable diseases including catecholaminergic polymorphic ventricular tachycardia (CPVT).

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Beta-Adrenergic Stimulation Increases the Intra-SR Ca Termination Threshold for Spontaneous Ca Release in Cardiac Myocytes

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In the heart, beta-adrenergic stimulation is associated with pro-arrhythmic Ca waves that occur as the result of the sarcoplasmic reticulum (SR) Ca content reaching a critical threshold level. Recently, we have shown that beta-adrenergic stimulation increases the intra-SR Ca threshold for Ca wave initiation, potentially serving as a protective mechanism against pro-arrhythmic Ca release during beta-adrenergic stimulation (Domeier et al., 2012). However, data regarding the termination of such release and details on the regulation of this process have yet to be elucidated. In this study we directly and dynamically measured the intra-SR Ca level ([Ca]SR) at which spontaneous Ca waves terminate (termination threshold) under control conditions and during beta-adrenergic stimulation. Application of the beta-adrenergic receptor agonist isoproterenol (ISO; 1 μM) resulted in an increase in basal [Ca]SR. Importantly, in the presence of ISO the [Ca]SR at which spontaneous Ca waves terminated was also increased compared to control conditions. In addition, the depletion amplitude of spontaneous Ca waves was decreased in the presence of ISO compared to control conditions. When [Ca]SR was subsequently lowered in the presence of ISO to that observed under control conditions (by reducing extracellular Ca and partially inhibiting SERCA with cyclopiazonic acid or thapsigargin), the [Ca]SR at which spontaneous release terminated was still increased compared to control conditions. Likewise, the depletion amplitude remained decreased compared to control conditions. These data indicate that during beta-adrenergic stimulation in the heart, both the intra-SR Ca threshold at which spontaneous Ca waves initiate and terminate is increased, while the amount of Ca released during Ca waves is decreased. The Ca wave termination level may represent an important mode of altering diastolic Ca wave amplitude, and thus, the arrhythmogenic potential of the cell during acute beta-adrenergic stimulation.

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InsP3-Induced Ca^{2+} Release in Atrial Myocytes

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The contribution and significance of $InsP_3$-induced Ca^{2+} release (IP3ICR) in cardiac excitation-contraction coupling (ECC) is still a matter of debate. IP3ICR may be involved and targeted during cellular remodeling of ECC under pathophysiological stress. Here we intend to characterize IP3ICR and the interplay of $InsP_3$Rs and RyRs in atrial myocytes. We hypothesized a functional crosstalk and/or cooperativity between both SR-Ca^{2+} release channels, e.g. IP3ICR may trigger or facilitate Ca^{2+}-induced Ca^{2+} release (CICR) via RyR activation and/or RyR sensitization. $InsP_3$-induced Ca^{2+} release events were activated by rapid superfusion with 20 μM $InsP_3$ in permeabilized atrial myocytes acutely isolated from mice hearts. Separation between CICR (Ca^{2+} sparks) and IP3ICR (Ca^{2+} puffs) was performed by pharmacological interventions using tetracaine and 2-APB. SR-Ca^{2+} release events were recorded using high-speed 2D confocal imaging. Data were analyzed using an IDL software based algorithm. Direct $InsP_3$R activation leads to an increase of 23% in the number of Ca^{2+} release events compared to the number of spontaneous events under control. 2D Ca^{2+} release event analysis revealed more Ca^{2+} events with smaller amplitudes (0.75, p=0.01) and increased FWHM (1.14, p=0.02) in the presence of $InsP_3$ compared to spontaneous Ca^{2+} sparks associated with RyRs openings. RyRs block (1 mM tetracaine) and/or $InsP_3$Rs inhibition (2 μM 2-APB) shift $InsP_3$ triggered local Ca^{2+} release events towards events with lower amplitude and higher FWHM which was not observed in the absence of $InsP_3$. This suggests that $InsP_3$ evoked SR-Ca^{2+} release events do not exist as singular $InsP_3$ evoked Ca^{2+} release events but as Ca^{2+} release events that derive from both RyRs and InsP3Rs and suggesting that IP3ICR may be linked to CICR in atrial cells. Supported by SNF.

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Efficient Automatic Analysis of High-Speed Confocal Images Containing Localized Calcium Release Events

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High-speed confocal microscopy is a relatively new method used to investigate localized calcium release events, most notably calcium sparks. Line scan images can reliably be recorded at speeds up to 60000 lines/s while for X-Y images 120 frame/s rate is achievable. The increased amount of recorded data raises the necessity of efficient automatic analysis methods. A set of images recorded on frog skeletal muscle cells after caffeine treatment was used to test the described methods.

We present a collection of methods useful for the automatic analysis of high speed line scan and X-Y recordings, mostly based on stationary wavelet transform (SWT). Our one-dimensional SWT based ember-detection method was adapted to detect sparks on high-speed line scan recordings. The center-line of detected sparks is automatically marked on each scan line to assess the spatial and temporal characteristics of the spark. 3.66% of all sparks (n=23704) in the test image set had complex spatial properties revealed by high-speed imaging. Amplitude and full width at half maximum (FWHM) is determined for each scan line to calculate the amount of calcium released (signal mass).

On X-Y image series two-dimensional SWT was used to denoise the images and to detect sparks on each frame. The orientation of the skeletal muscle cell and the position of sarcomeres are determined using fast Fourier-transform on the stationary components of the image series. The position of the center for each spark is determined relatively to the sarcomeres. FWHM is calculated in the axes perpendicular ($FWHM_X$) and parallel ($FWHM_Y$) to the sarcomeres. For the test dataset (n=22426), $FWHM_X/FWHM_Y$ is 1.22.

The above-described methods enable us to automatically analyze high-speed confocal images and to reveal some of their properties which were hidden on conventional confocal images.

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Multiscale Model of the Propagation of the Fertilization Wave in Sea Urchin Eggs

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We present a multiscale model describing blocking polyspermy during the fertilization in sea urchin eggs [1,2]. Our goal is to describe the processes controlling the formation of the vitelline envelope as a consequence of hyaline exocytosis. Our model combines two different levels of description: a) A reaction-diffusion scheme based on the CICR model [3], accounting for the initial propagation of the calcium wave, its duration and spatial distribution in the cell. b) The activation and dynamics of kinesin motors transporting vesicle clusters that contain hyaline, triggered due to calcium signaling. Our model incorporates a recent biochemical model [4] describing the kinetics of kinesin processivity in order to reproduce observed translational velocities, their duration and dependence on local ATP concentration. In addition, this dynamics is incorporated into a diffusion equation in the presence of external forces that accounts for the motions of vesicle clusters along the citoskeletal filaments of the egg [5]. Our model reproduces very well experimental data associated to the propagation velocities of the calcium wave, the kinesins and the vesicle clusters, as well as their duration and the kinetics of fluorescence used to indirectly quantify the cumulative exocytosis of hyaline at the plasmatic membrane.

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